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Effects of spray freeze drying and pulsed electric fields on fenugreek seed extract properties

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ABSTRACT

Plant extracts are increasingly recognized for their potential health benefits, creating a growing demand for efficient extraction and preservation methods. In this study, a pulsed electric field (PEF) system was applied to maximize the extraction of plant bioactives, followed by encapsulation using spray freeze drying (SFD), a novel preservation technique. Among solvents tested, ethanol yielded the highest extract recovery (166.66 mg GAE/g), whereas methanol (sample-to-solvent ratio 1:10) resulted in the highest total polyphenol content. In the encapsulation process, gum acacia (GUA) at a 1:6 extract-to-binder ratio with a 30-hour lyophilization period produced powders with the lowest moisture content (3.9% w.b.), and SFD particles exhibited an average size of 9.0 µm. Furthermore, GUA as a binder and guar gum at a 20-hour lyophilization period achieved the highest polyphenol encapsulation efficiency. These findings highlight PEF-assisted extraction combined with SFD encapsulation as a cost-effective and efficient strategy for industrial-scale preservation of plant bioactives.

Key words: Pulsed electric field, spray freeze drying, encapsulation, gum acacia, plant extracts.

INTRODUCTION

The extraction and stabilization of essential oils and plant-derived bioactives remain major challenges in food and pharmaceutical industries. Conventional solvent extraction, though widely employed, suffers from several limitations such as long processing time, low extraction efficiency, residual solvents, loss of volatile compounds, and high energy consumption (Mazicioglu, 1). These drawbacks not only reduce yield and quality but also raise concerns about safety, sustainability, and environmental impact. Consequently, there is a growing demand for green and energy-efficient technologies that can achieve higher recovery of bioactive compounds while preserving their functional integrity.

Pulsed electric field (PEF) technology has emerged as a promising alternative to conventional extraction. PEF involves the application of short, high-intensity electric pulses (up to 30 kV) over a very short duration (µs), leading to electroporation and permeabilization of plant cell membranes (Siddeeg *et al.,* 2; Arshad *et al.,* 3). This facilitates enhanced mass transfer, resulting in improved release of intracellular components. The technique has attracted industrial interest due to its non-thermal, energy-efficient, and environmentally sustainable nature, which enables the recovery of sensitive bioactives without thermal degradation (Zhang *et al.,* 4). Beyond extraction, stabilization of bioactive compounds during downstream processing and storage is equally critical, as many natural metabolites are prone to degradation when exposed to oxygen, light, or high temperatures. Encapsulation technologies provide an effective strategy to overcome these challenges, offering protection, controlled release, and improved solubility of bioactives. Various techniques are available, including co-extrusion, spray drying, spray chilling, bead processing, and spray freeze drying (SFD) (Carneiro *et al.,* 5). Among these, SFD stands out as a low-temperature technique (Mumenthaler and Leuenberger, 6), capable of producing particles with uniform size distribution (Chai *et al.,* 7), high porosity, and rapid redispersion in aqueous systems (Christie *et al.,* 8). These advantages make SFD particularly suitable for encapsulating thermolabile compounds.

Given these considerations, the integration of advanced extraction and encapsulation strategies offers a promising approach to maximizing yield and stability of plant-derived bioactive. Therefore, the present study was undertaken to overcome the disadvantages of conventional methods by optimizing pulsed electric field extraction for enhanced recovery of fenugreek seed extract and employing spray freeze drying for effective encapsulation, ensuring maximum retention, stability, and functionality of bioactive compounds.

MATERIALS AND METHODS

One kilogramme of *Gujarat Methi-2* variety fenugreek seeds was soaked in water for 12 hours, then spread out in a seed germinator set at 25°C and

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90% relative humidity for three days. seeds were then dried in a hot air oven at 55°C until moisture content was 7% (w.b.). total polyphenolics were estimated by a method described by Nakpanich *et al*. (9) and Ishwarya *et al*. (10) and standard curve obtained in Figure 1. Following formula used to calculate yield of extract.

$$\text{Yield (\%)} = \frac{W_1}{W_2} \times 100$$

Where W_1 refer to weight (g) of extract residue obtained after solvent removal and W_2 refer to weight (g) of fenugreek seed powder.

Minerals content were analyzed through ICP-OES (Inductively Coupled Plasma – Optical Emission Spectroscopy) (Model Number 7000 DV, Perkin Elmer USA).

The germinated seed powder passed through 60-mesh sieve used for extraction using PEF-system (ENERGY PULSE SYSTEM - PORTUGAL). distance maintained between two parallel electrode plates was 00010 cm. Interactions between independent variables at actual levels and its responses were studied using factorial completely randomized design (FCRD). ANOVA was conducted on experimental data and significance of pulsed electric voltage (5 kV, 10 kV and 15 kV), types of solvent (33 % ethanol and 33 % methanol), sample to solvent ratio (1:8, 1:10 and 1:12) on yield of extract (%) and an amount of TPP (mg GAE/g). Extract for SFD was prepared from optimized method of PEF-assisted extraction. This extract was stored in glass vials at -20°C and used as and when required. Guar gum (GG), whey protein isolate (WPI), gum arabic (GA), and soy protein isolate (SPI) were binding materials employed. By making emulsions with 10 g of binding material dissolved in 50 ml of water, stability of emulsions was verified. Emulsions were given three hours to stand in 15 ml test tubes before separation was visually assessed and selected best. In SFD process, extract was freeze dried at three different levels of drying times (20, 25 and 30 h). atomizer pressure kept between 2.0 kg/cm^2. Throughout SFD process, feed pump flow rate of 0.6 ml/min and stirrer speed of 72 rpm were maintained. Process optimized using factorial completely randomized design in design expert® (version 11) software. Moisture content of samples was determined as per method described by AOAC (11). Encapsulated powder analysed for particle size using Biovis system (PSA2000). Encapsulation efficiency according to procedure given by Jafari *et al*. (12). Encapsulated powder prepared by SFD was further evaluated for powder properties and were measured based on procedure given by Asif-Ul-Alam *et al*. (13) and Jinapong *et al*. (14). Morphology of encapsulated powders analyzed by Scanning Electron Microscope (XL 30 ESEM with EDAX: Resolution:upto 2; Accelerating voltage: 30 kV; Magnification: upto 2,50,000x).

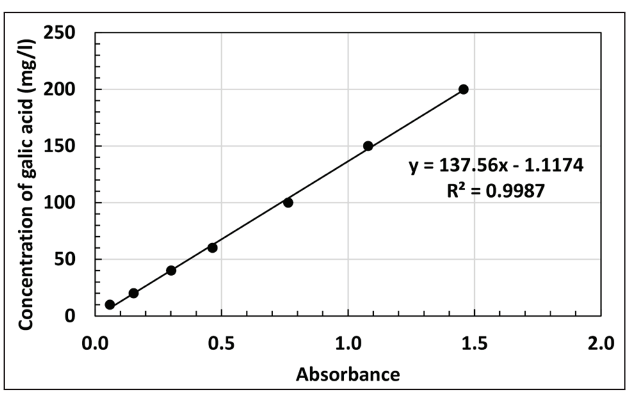

Fig. 1. Standard curve of gallic acid.

RESULTS AND DISCUSSION

The polyphenol rich extract yield increased when PEF-voltage increased (Table 1). solvent, ethanol showed maximum extract yield than methanol. Maximum FSE yield (10.89 %) observed in treatment

Table 1: Effect of pulsed electric field-assisted extraction parameters on extract yield and recovery of total polyphenols.

Treatment	Voltage (kV)	Solvent	Sample: Solvent Ratio	Yield (%)	TPP (mg GAE/g)
P_1	5	Ethanol	1:8	6.94	118.00
P_2	10	Ethanol	1:8	9.53	158.00
P_3	15	Ethanol	1:8	8.93	97.66
P_4	5	Ethanol	1:10	7.49	134.33
P_5	10	Ethanol	1:10	8.47	166.66
P_6	15	Ethanol	1:10	9.97	93.66
P_7	5	Ethanol	1:12	6.94	115.00
P_8	10	Ethanol	1:12	10.89	148.00
P_9	15	Ethanol	1:12	8.86	140.00
P_{10}	5	Methanol	1:8	7.04	118.33
P_{11}	10	Methanol	1:8	7.82	146.66
P_{12}	15	Methanol	1:8	9.39	124.66
P_{13}	5	Methanol	1:10	7.10	126.00
P_{14}	10	Methanol	1:10	8.47	154.66
P_{15}	15	Methanol	1:10	8.27	88.33
P_{16}	5	Methanol	1:12	6.94	123.33
P_{17}	10	Methanol	1:12	8.03	138.00
P_{18}	15	Methanol	1:12	8.61	89.66
C.V (%)	-	-	-	14.70	0.874

of 10 kV voltage with ethanol and 1:12 sample to solvent ratio. Puertolas *et al.* (15) concluded that PEF-is low energy (8.92 kJ/kg) process consuming extraction method compared to conventional methods while working on anthocyanin rich extracts. Increase in FSE yield while using PEF might be due to electroplasmolysis of cell membranes and electroporation effect (Goettel *et al*., 16). Results however showed that all linear and interactive factors had non-significant effect ($p< 0.05$) on extract yield (%) at 5 % level of significance with C.V value of 14.7. Table 1 shows that voltage, solvent and sample to solvent ratio had significant effect on total polyphenolics at 5 % level of significance. With an increase in PEF voltage, TPP rose noticeably. Ethanol had a higher TPP level than methanol, and as sample to solvent ratio rose. Highest TPP (166.66 mg GAE/g) were observed in treatment of 10 kV voltage, ethanol with 1:10 sample to solvent ratio. Similar results were reported in citrus fruits, *Laminaria digitata* and *Momordica charantia* L. (El Kantar *et al.,* 17; Einarsdottir *et al.,* 18; Einarsdottir *et al.,* 19). In order to optimize extraction procedure, three dependent factors mentioned above (extract yield and total polyphenols) were taken into account. Three factorial CRD statistical analysis and Design-Expert@7.0.0 programme was used for optimization. Based on statistical data, best settings for extraction procedure were ethanol as solvent, PEF-assisted extraction method with 10 kV voltage, and a 1:12 sample to solvent ratio. These results showed highest TPP (116.66 mg GAE/g) and maximum extract yield (10.89%). It was found that encapsulated powder yield increased when extract to binder ratio increased in case of GUA and GA with GG as binders. Among 20, 25 and 30 h lyophilization times, 30 h yielded maximum encapsulated powder. Yield was high in gum acacia (GUA) binder than GUA with GG binder followed by WPI. When extract to binder ratio increased, lyophylization time also increased. Similar results were reported by Maa *et al.* (20) in protein powder by using both SFD and spray drying. All linear and interactive factors had significant effect ($p< 0.05$) on encapsulated powder yield (%) at 5% level of significance with C.V value of 2.12. WPI-based powder has greatest moisture content ever seen because of its strong hydrogen bonding capacity (Table 2) viscosity of binding materials increased with thermal conductivity. Encapsulated powder made using GUA binder, a 1:5 extract to binder ratio, and a 30-hour lyophilization duration in a freeze dryer had lowest moisture content (3.96 percent w.b.). Maa *et al.* (20) observed similar outcomes. SEM micrographs revealed that the guar gum matrix (GUA, 7.5:2.5) exhibited a compact and smooth surface whereas spray freeze-dried fenugreek seed powders showed highly porous, irregular structures with increased surface area, indicating effective encapsulation of bioactive compounds (Fig. 2). Particle size is inversely proportional to hygroscopicity which in turn causes agglomeration (lumps formation) under humid conditions (Table 2). It was found that when extract to binder ratio (from 1:4 to 1:6) increased, particle size also increased. Particle size increased when lyophilization time (from 20 h to 30 h) increased. Compared to GUA and WPI binders, GUA with GG binder was found with larger size particle size and lowest particle size (41.67 μm) was observed in treatment of GUA powder, 1:4 extract to binder ratio and 20 h lyophilization time. Lower sample size will drastically reduce drying time because SFD time varies with square of particle

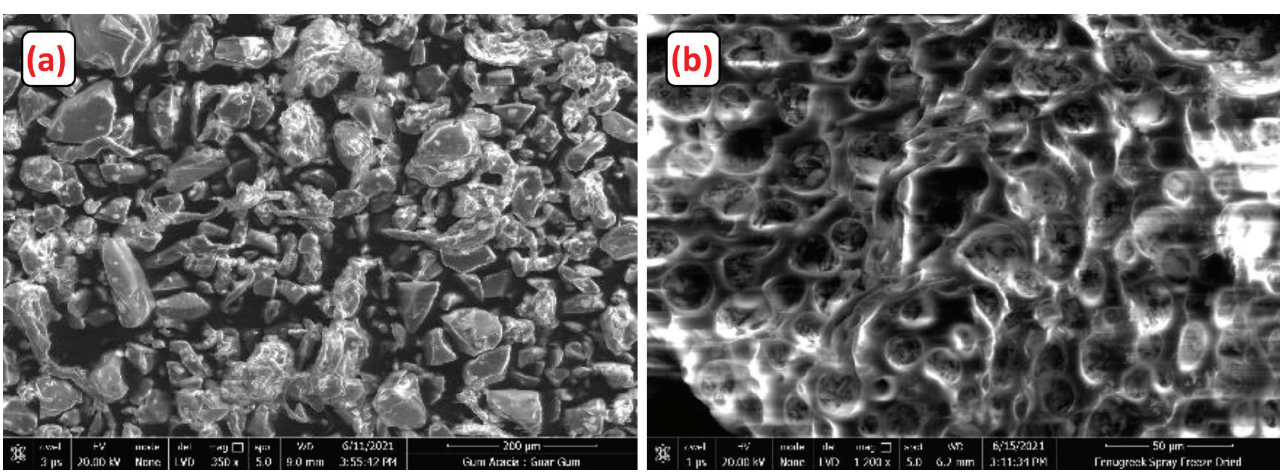

Fig. 2. SEM images of powders; a) GUA:Guar gum (7.5:2.5) ; b) Spray freeze dried powder of bioactive compounds from fenugreek seeds.

Table 2: Effect of spray freeze drying on encapsulation characteristics of bioactive compounds from fenugreek seeds.

Treatment	Binder	Extract: Binder	Lyophilization time (h)	Yield (%)	Moisture (%)	Particle size (µm)	Polyphenolis EE (%)	Total polyphennols (mg GAE/g)
T_1	GA	1:5	20	87.11	4.44	42.67	74.50	72.41
T_2	GA	1:5	25	87.16	4.37	46.03	76.93	50.62
T_3	GA	1:5	30	84.91	3.96	44.36	78.48	79.08
T_4	GA	1:4	30	82.18	4.15	46.89	78.26	80.63
T_5	GA	1:6	20	86.32	4.49	43.80	75.52	72.28
T_6	GA	1:4	20	84.39	4.42	41.67	74.64	71.44
T_7	GA	1:4	25	87.11	4.51	45.59	47.91	52.69
T_8	GA	1:6	25	85.97	4.45	44.07	46.51	48.89
T_9	GA+GG	1:6	25	84.51	4.78	51.86	78.33	73.15
T_{10}	GA+GG	1:4	30	88.29	4.43	52.08	73.62	65.17
T_{11}	GA+GG	1:4	20	84.08	4.95	48.45	81.60	74.49
T_{12}	GA+GG	1:5	30	82.47	4.42	52.84	75.35	64.06
T_{13}	GA+GG	1:4	25	81.91	4.81	50.44	77.86	72.49
T_{14}	GA+GG	1:5	20	82.45	4.80	47.88	83.42	74.44
T_{15}	GA+GG	1:6	20	86.45	4.87	48.66	81.99	74.96
T_{16}	GA+GG	1:6	30	84.94	4.58	50.91	73.26	64.19
T_{17}	GA+GG	1:5	25	79.71	4.50	51.62	79.14	72.96
T_{18}	WPI	1:5	30	72.57	5.44	50.32	27.70	53.00
T_{19}	WPI	1:6	30	70.58	5.33	47.43	28.20	53.65
T_{20}	WPI	1:6	25	78.66	5.92	48.76	21.66	53.84
T_{21}	WPI	1:4	20	78.75	5.53	47.73	24.46	58.27
T_{22}	WPI	1:5	25	81.36	5.73	48.59	21.93	54.16
T_{23}	WPI	1:5	20	74.91	5.68	44.82	22.50	56.52
T_{24}	WPI	1:4	25	75.05	5.66	51.45	22.22	54.10
C.V (%)	-	-	-	2.12	5.44	2.75	2.02	3.34

Where GA-GUA, GG-Guar gum, WPI-Whey protein isolate

thickness (Parthasarathi, and Anandharamakrishnan, 21). particle size 41.67 µm was observed higher than results reported by Maa *et al.* (20) and Costantino *et al.* (22). High polyphenol EE gives high amount of bioactive compounds retention (Table 2). GUA with GG binder gave maximum polyphenol EE (83.42%) compared to GUA (78.48%) and WPI (28.20%) binders. EE is inversely proportional to extract to binder ratio. Polyphenols were not much affected by lyophilization time. However, as lyophilization time increased, EE decreased in WPI binders. Highest polyphenol EE (83.41%) was observed in treatment of GUA with GG binder, 1:5 extract to binder ratio and 20 h lyophilization time. Kumar *et al.* (23) reported that EE with GUA of fermented tea leaf waste water's bioactive compounds by freeze drying was 98.05 percent. Among binders applied, GA was found with maximum TPP than other two binders. When extract to binder ratio increased TPP were decreased in WPI encapsulated powder. Highest TPP (80.63 mg GAE/g) was observed in GUA with 1:4 extract to binder ratio lyophilized for 30h. Optimization was done by using statistical analysis by three factorial CRD and Design-Expert@ 7.0.0 software. Optimized encapsulation process was with GUA as binder, 1:5 extract to binder ratio, 20 h lyophilization time in which maximum yield (87.10 %), minimum moisture content (4.44 % w.b) and maximum polyphenols EE (74.50 %) were observed. Optimized conditions for SFD are shown in Table 3 results were validated by conducting experimental optimized conditions wherein results showed a marginal variation of 2.1 %. data related to characteristics of spray freeze dried encapsulated powder is depicted in Table 4. Bulk density, true density porosity and Carr index of spray freeze-dried powder were 0.54 g/cm^3, 0.57 g/cm^3,

Table 3: Optimized conditions for spray freeze drying of bioactive compounds from fenugreek seeds.

Parameter	Value (Mean)
Binder	GUA
Extract to binder ratio	01:05
Processing condition (lyophilization time h.)	20.00
Moisture (%)	04.44
Yield (%)	87.10
Polyphenols Encapsulation efficiency (%)	74.49

Table 4: Characteristics of spray freeze dried encapsulated powders of bioactive compounds from fenugreek seeds.

Character	Value
Bulk density (g/cm^3)	0.54 ± 0.01
True density (g/cm^3)	0.57 ± 0.05
Porosity (%)	4.11 ± 0.32
Carr Index	10.0 ± 0.24
Solubility (%)	87.6 ± 1.72
Wettability (min:sec)	5:50 ± 0.00
Hygrscopicity (g/kg dry solids)	3.12 ± 0.06
Dispersibility (%)	71.0 ± 1.56
Water activity	0.36 ± 0.01

4.11 % and 10 respectively. Solubility, wettability, hygrscopicity, dispersibility and water activity values of spray freeze dried powder were 87.6 %, 5:50 min:sec, 3.12 g/kg dry solids, 71 ± 1.56 % and 0.36 ± 0.01. Similar results of physico-chemical properties were reported in spray dried fruit juices (Kalita *et al.,* 24). GUA: Guar gum (7.5:2.5) powder exhibited sharp corner surfaces without porous nature whereas spray freeze dried microcapsules exhibited irregular flake like structures with a porous nature. Flake like porous structures might be due to direct sublimation of ice into water vapor during freeze-drying operation. Spray freeze-dried microcapsules were spherical and uniform in size, which was similar to morphology study of whey protein microcapsules reported by Parthasarathi and Anandharamakrishnan (21). From results, it can be interpreted that higher wettability and dispersibility can be obtained in SFD fenugreek seed extract. From EDAX analysis (Fig. 3, Fig. 4 and Table 5) it is observed that toxic metal compounds such as Cu, Pb, Cd, Hg and As are absent and hence this encapsulated powder is considered as safe for consumption.

The highest extract yield (10.89%) in PEF-assisted extraction procedure was seen at a voltage of 10 kV and a sample to solvent ratio of 1:12. Treatment with highest TPP (166.66 mg GAE/g) at a ratio of 1:10 sample to solvent and 10 kV voltage was noted. Encapsulated powder made from GUA binder, with a 1:6 extract to binder ratio and a 30-hour lyophilization duration, had lowest moisture content (3.9%) in SFD. Powders derived from SFD have a

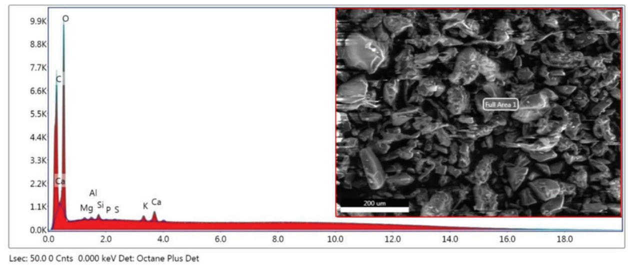

Fig. 3. Energy dispersive X-ray analysis (EDAX) spectrum of wall material (GUA:Gaur gum=7.5:2.5) sample. Scale bar: 200 µm.

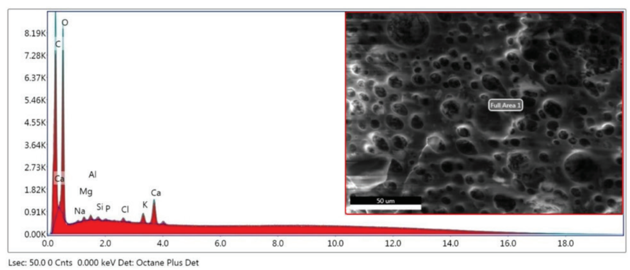

Fig. 4. Energy dispersive X-ray analysis (EDAX) spectrum of spray freeze dried sample of bioactive compounds from fenugreek seeds. Scale bar: 50 µm.

Table 5: EDAX analysis of wall material and spray freeze dried samples of bioactive compounds from fenugreek seeds.

Element	Wall material (GA:GG = 7.5:2.5) sample		Spray freeze dried sample	
	Weight %	Atomic %	Weight %	Atomic %
C	36.04	44.21	33.78	41.61
O	57.84	53.27	60.82	56.25
Mg	0.24	0.15	0.13	0.08
Al	0.33	0.20	0.11	0.06
Si	0.32	0.18	0.38	0.20
P	0.17	0.09	0.18	0.09
Cl	0.06	0.03	0.20	0.08
K	0.11	0.05	1.23	0.46
Ca	0.32	0.13	3.18	1.17

Where GA-GUA, GG-Guar gum, C-Carbon, O-Oxygen, Mg-Magnesium, Al-Aluminium, Si-Silicon, P-Phosphorous, Cl-Chlorine, K-Potassium, Ca-Calcium particle size of 9 µm. treatment of GA with GG binder, 1:5 extract binder ratio, and 20-hour lyophilization duration produced highest polyphenol EE (83.4%). Therefore, spray freeze drying is a time- and money-saving method that may be further investigated to provide novel, creative ideas for effective distribution of bioactive chemicals in development of functional and nutraceutical food formulations in food industries.

AUTHORS' CONTRIBUTION

Conceptualization of Research, Designing of the experiments, Contribution of Experimental materials and Execution of field/ lab experiments, data collection (BP); Analysis of data and interpretation, interpretation of manuscripts (RVP).

DECLARATION

The authors do not have any conflicts of interest to declare.

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